Little, Judith Paige and Tevelen, Gregory and Adam, Clayton J. and Evans, John H. and Pearcy, Mark J. (2009) *Development of a biaxial compression device for biological samples : preliminary experimental results for a closed cell foam.* Journal of the Mechanical Behavior of Biomedical Materials, 2(3). pp. 305-309.

TITLE:

Development of a biaxial compression device for biological samples: Preliminary experimental results for a closed cell foam

AUTHORS:

J P Little[1,2], G Tevelen[2], C J Adam[1,2], J H Evans[1,2], M J Pearcy[1,2]

AFFILIATION:

[1] School of Engineering Systems, Queensland University of Technology, Brisbane, Australia

[2] Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

CORRESPONDING AUTHOR:

Dr J Paige Little

BEE Research Portfolio

O Block – Level 7

Gardens Point Campus

2 George Street

Brisbane QLD 4000

AUSTRALIA

Ph: +61 7 3138 5112

Fax: N/A

Email: j2.little@qut.edu.au

JOURNAL

Journal of the Mechanical Behavior of Biomedical Materials

Submission Type

Technical Brief

ABSTRACT

Biological tissues are subjected to complex loading states *in vivo* and in order to define constitutive equations that effectively simulate their mechanical behaviour under these loads, it is necessary to obtain data on the tissue's response to multiaxial loading. Single axis and shear testing of biological tissues is often carried out, but biaxial testing is less common. We sought to design and commission a biaxial compression testing device, capable of obtaining repeatable data for biological samples. The apparatus comprised a sealed stainless steel pressure vessel specifically designed such that a state of hydrostatic compression could be created on the test specimen while simultaneously unloading the sample along one axis with an equilibrating tensile pressure. Thus a state of equibiaxial compression was created perpendicular to the long axis of a rectangular sample. For the purpose of calibration and commissioning of the vessel, rectangular samples of closed cell ethylene vinyl acetate (EVA) foam were tested. Each sample was subjected to repeated loading, and nine separate biaxial experiments were carried out to a maximum pressure of 204 kPa (30psi), with a relaxation time of two hours between them. Calibration testing demonstrated the force applied to the samples had a maximum error of 0.026N (0.423% of maximum applied force). Under repeated loading, the foam sample demonstrated lower stiffnesses during the first load cycle. Following this cycle, an increased stiffness, repeatable response was observed with successive loading. While the experimental protocol was developed for EVA foam, preliminary results on this material suggest that this device may be capable of providing test data for biological tissue samples. The load response of the foam was characteristic of closed cell foams, with consolidation during the early loading cycles, then a repeatable load-displacement response upon repeated loading. The repeatability of the test results demonstrated the ability of the test device to provide reproducible test data and the low experimental error in force demonstrated the reliability of the test data.

KEYWORDS

Biaxial compression, pressure vessel, biological tissue testing

NOMENCLATURE

Q = Volume flow rate;

l = circumference of the piston;

a = clearance between piston and bore;

Δp = pressure variation;

μ = viscosity;

L = length of bore;

τ, = shear stress;

u = fluid velocity;

Δp = pressure variation along piston length;

mM = millimolar

INTRODUCTION

Biological tissues demonstrate complex mechanical behaviour under three dimensional loading states. With the increasing prevalence of computational and analytical models to simulate biological systems, there is an increasing need to accurately represent this behaviour using more advanced constitutive models. These models must be capable of capturing such tissue responses as anisotropy, hyperelasticity, viscoelasticity and/or poroelasticity. In order for these models to capture this behaviour, detailed experimental data on the multiaxial response of the tissue is necessary (Sacks and Sun 2003).

Sacks and Sun (Sacks and Sun 2003) state that for incompressible materials, biaxial mechanical data is ideal for determining the parameters governing three dimensional tissue constitutive equations. These researchers propose specific features which should be present in a biaxial testing device for it to provide accurate data, with minimal testing artefact. These features include:

- Unhindered lateral expansion, in the off-load-axis direction;
- Generation of a uniform strain state centrally in the sample, for strain measurement;
- Strain measurements made remote from specimen grips to avoid edge artefacts; and
- Strain measurements made optically, to avoid any mechanical interference from measuring devices.

Previous researchers have demonstrated the biaxial response of soft tissues, such as skin, lung and arteries, using biaxial tension testing on cruciform-type samples (Fronek *et al.* 1976; Lanir and Fung 1974; Zeng *et al.* 1987). However, this testing method relies on acquiring test samples of a sufficient size and aspect ratio to avoid edge effects and furthermore, biaxial-tensile test results may be biased by bridging fibre response in highly collagenous biological tissues. Of particular interest in the current study, is the acquisition of biaxial experimental data for the intervertebral disc anulus ground matrix. In determining the biaxial properties for this tissue, the biaxial-tension test response would be dominated by the stretching of the embedded collagen fibres, whereas under compressive loading this does not occur, allowing determination of the constitutive response of the anular ground matrix.

We are aware of only one other group who have investigated the biaxial response of the anulus fibrosus (Bass *et al.* 2004), however this was under tensile loading. Arguably, the intervertebral disc and specifically, the anulus ground matrix are exposed to both tensile and compressive load states during physiological activities.

As a first step in deriving a comprehensive set of data defining the response of the anulus ground matrix to three dimensional loading, this study aimed to develop, commission and conduct preliminary experiments using a biaxial compression testing device.

METHODS

A novel testing rig was designed and built to carry out biaxial compression. The design objective for the rig was to apply a hydrostatic compressive pressure to a specimen, while simultaneously unloading it along one axis to obtain a state of biaxial compression. The rig was designed for testing of biological tissues, but for the purpose of calibration and commissioning, rectangular samples of closed cell ethylene vinyl acetate (EVA) foam were employed and data for this will be presented. EVA foams are known to demonstrate consolidation under a preconditioning load, followed by a repeatable force-displacement response upon repeated loading (Nusholtz *et al.* 1996).

Design and principle of operation

The testing rig comprised a stainless steel rectangular vessel, which was filled with Ringers' solution (116mM NaCl, 1.2mM KCl, 1.0mM $CaCl_2$, 2.7mM $NaHCO_3$ in 1L $H_2$0) and pressurised (Figure 1). The principal of operation of the biaxial testing device is outlined schematically in Figure 2. Two viewing windows (19mm thick standard glass plugs) were inserted in two opposite walls of the vessel and pressure sealed with an O-ring. The remaining walls provided attachment sites for two pieces of durable nylon thread (Figure 1 B, Figure 3 B,C), the ends of which were glued to the end surfaces of the foam specimen (Figure 3 C). Loctite ® 401 (Henkel Australia Pty Ltd) cyanoacrylate adhesive was used to bond these faces. Thus the specimen was suspended in the centre of the vessel and could be viewed through the windows. When the pressure to the vessel was increased, this pressurised a 10mm air gap at the top of the sealed vessel and in turn pressurised the solution.

One of the pieces of nylon was attached to a press-fit insert in one wall. This insert could be rotated from outside the vessel, to control specimen orientation. The other piece was attached to the end of a glass ceramic piston running in a well polished bore in the opposite wall of the vessel (Figure 3). The cross-sectional area of the piston was the same as the surface area of the specimen end ($9mm^2$) to which it was connected, thus equilibrating the compressive force along the long axis of the specimen. As such, there was no compressive force acting on the specimen in the axis of the piston and the compressive force in the other two transverse directions was unaffected. A rectangular foam sample with a square cross-section of 3.5x3.5mm and length of 10mm was used. (It was not possible to make a specimen of this material of the required 3x3 mm cross-section but for the purpose of assessing the function of the device this was adequate.)

With increasing compressive pressure on the specimen, the transverse dimensions reduced and due to Poisson’s effect, the long axis dimension increased. Therefore, it was necessary that the piston move within the bore, maintaining tension in the nylon thread. As such, a key design feature was that at pressures exceeding gauge, the fluid was able to leak from the vessel through a precisely machined clearance between the piston and bore. This clearance was calculated using the theory of laminar flow of fluids between two parallel plates (Eqn 1) and ensured that for the duration of a test, the flow rate did not deplete the fluid volume in the vessel below the level of the suspended specimen.

$$\frac{Q}{l} = \frac{a^3.\Delta p}{12.\mu.L}$$

Eqn 1 Theory of laminar flow between parallel plates. In this case, Q = Volume flow rate, l = circumference of the piston, πD , a = clearance between piston and bore, Δp = pressure variation, μ = viscosity of Ringers solution, L = length of bore.

The piston was manufactured from Macor Machinable Glass® glass ceramic and the low piston weight allowed it to be suspended on a layer of fluid when the pressure in the vessel was increased. The polished finish on the bore and piston surfaces and the use of Ringers' solution as lubricant ensured there was very low frictional resistance between bore and piston. A pressure inlet in the lid of the vessel was connected to an air compressor through a high precision pressure regulator (Model:11-818, IMI Norgren Ltd, Staffordshire, UK, Max Press: 408 kPa (60psi), Accuracy: 3 kPa (0.435psi)) which ensured accurate control of the pressure in the vessel.

The vessel height was determined to ensure the weight of fluid above the specimen did not generate a high prestress. The maximum head of fluid above the specimen exerted a pressure of 0.4 kPa which was considered negligible.

Commissioning and proof testing of the device

The vessel was designed in accordance with Australian Standard AS1210-1997. A design pressure of 1.03MPa (150psi) was used, which included a safety factor of 2.5. The standard prescribed the design material strength, the minimum wall thickness, the requirement for a pressure relief valve and the need for proof testing. Proof testing was carried out at twice the design pressure or 2.06 MPa (300psi) for 30 seconds and the vessel assessed for any visible deformation or leakage.

Data measurement

Measurement of the biaxial pressure in the vessel during testing was achieved using a Druck pressure calibrator (DPI 705, GE Druck Ltd, Leicester UK). Deformation of the specimen under load was measured using a Sigmascope 300 Shadowgraph profile projector (Herbert Controls and Instruments Ltd, Letchworth, UK) whereby a light source was directed through the viewing windows, projecting the shadow of the deformed specimen onto a calibrated viewing screen and allowing measurement of the specimen deformed width (image magnification was accounted for during machine setup) with an accuracy of 0.001mm.

Pressure vessel calibration

To ensure the force acting on the inner face of the piston was accurate, the vessel was assembled with the outer face of the piston in contact with a 500N Hounsfield load cell (Hounsfield Test Equipment, Red Hill, England). Fluid in the vessel was incrementally pressurised and the force output from the load cell recorded. Five sets of pressure measurements were obtained at pressures between 0 and 659 kPa (97 psi). The calculated force (based on fluid pressure and piston cross-sectional area) was compared with the Hounsfield measured force minus the wall shear stress due to fluid flow through the bore-piston clearance. The shear stress was calculated at specific fluid pressures using Eqn 2.

A $$\tau = \mu \frac{\partial u}{\partial y}$$

B $$\frac{\partial u}{\partial y} = \frac{1}{2\mu} \cdot \left(\frac{\Delta p}{l} \right) \cdot (2y - a)$$

Eqn 2 A. Shear stress, τ, as a function of fluid velocity, u, and the relative distance, y, measured across the clearance between the piston and bore, a. B. Velocity profile for fluid flow between infinite parallel plates (μ = viscosity, Δp = pressure variation along piston length)

Biaxial compression of EVA foam

A rectangular sample of closed cell EVA foam was tested to determine the repeatability of the testing technique. During eight separate biaxial experiments the test piece was loaded to a maximum pressure of 204 kPa (30psi) in increments of 34 kPa (5psi). The specimen was permitted to relax for two hours between tests. The deformation at each pressure was assessed by recording the minimum transverse width of the test piece. The deformation was normalized with the original specimen width, measured at gauge pressure.

RESULTS

Proof testing

At 1.03 MPa (150psi) and 1.53 MPa (225psi), the condition of the vessel was assessed – there was no visible leakage and all components were undeformed and intact. At 2.06 MPa (300psi) there was very minimal leakage from the fasteners in the lid, but this was eliminated with tightening of the screws. Following this pressurisation test, the vessel was considered safe for further use.

Calibration tests

The average error between the calculated force and the corrected measured force was 0.22% of the corrected value (Table 1). This error tended to increase with increasing pressures, to a maximum of 0.026N at 659kPa (97psi) which was 0.423% of the maximum corrected force.

Biaxial compression testing of EVA foam

The foam exhibited lower stiffness (Figure 4a) and deformations (Figure 4b) during the first load cycle compared to the remaining cycles. Stiffness was calculated as the slope of the secant joining the first and last datapoints on the pressure-strain response (Figure 4a). Data for cycle two was not used for data analysis due to an operator error in aligning the sample parallel to the plane of the viewing window. During cycles three to nine, the foam response demonstrated a repeatable behaviour upon successive loading (Figure 4).

DISCUSSION

An experimental device for biaxial compression testing of rectangular samples was developed and tested. This device comprised a pressure vessel, designed and proof tested in keeping with Australian Standards and according to AS4343-1999, carried a hazard level 'E' which was classified as 'negligible' risk.

Since the principal of operation for this biaxial compression device relies on equilibration of the hydrostatic force applied to the faces of a hexahedral testing sample, if the biological tissue tested is an open-pore structure, fluid flow through these pores could potentially serve to reduce the pressure applied to the longitudinal sides of the sample. As such, this testing device would not be appropriate for biaxial testing of open-pore biological tissues (eg. Trabecular bone). This device was designed in order to test specimens from the anulus fibrosus of intervertebral discs at strain rates comparable to physiological loading. At such loading rates it has been shown that the low porosity of cartilagenous tissues does not permit fluid movement to occur in the timescale of the strain application, resulting in the tissue behaving as an incompressible material (Higginson *et al.* 1976).

Currently, the test device requires samples with a cross-sectional area of exactly 9mm^2 in order for the axial force along the specimen to be equilibrated with the force acting on the piston. While the testing protocol was commissioned using samples of closed cell EVA foam which could not be manufactured to this specific dimension, it is intended for the testing of biological soft tissue samples which can be harvested with regular cross-sectional dimensions (eg samples with bony end attachments or cartilage). It was considered that the use of a slightly oversized sample cross-section did not detract from the demonstrated repeatability and reproducibility of the test results obtained with the device. It is also possible to manufacture a series of pistons and bores to accommodate other cross-sectional dimensions.

The EVA foam samples exhibited lower stiffness during the first cycle, then an increased stiffness, repeatable response upon successive loading. This behaviour is characteristic of the preconditioning behaviour of foams (Nusholtz *et al.* 1996), and the repeatability of test data suggested that the device and testing protocol were capable of providing accurate and reproducible experimental data. Results of the calibration testing showed a sufficiently low error in the applied force on the piston (<1%), to indicate reliability in the experimental results.

In future studies, the biaxial compression vessel will be utilised to measure the biaxial response of the anular soft tissues of the intervertebral disc. Measuring the response of spinal soft tissues to multiaxial loading states is important for understanding tissue behavior *in vivo*, and these preliminary results on EVA foam suggest that this device is capable of providing test data suitable for direct input to computational models of spinal motion segments (Little *et al.* 2007).

REFERENCES

Bass, E. C., Ashford, F. A., Segal, M. R.,Lotz, J. C., 2004. Biaxial testing of human annulus fibrosus and its implications for a constitutive formulation. Ann Biomed Eng 32(9): 1231-42.

Fronek, K., Schmid-Schoenbein, G.,Fung, Y. C., 1976. A noncontact method for three-dimensional analysis of vascular elasticity in vivo and in vitro. J Appl Physiol 40(4): 634-7.

Higginson, G. R., Litchfield, M. R.,Snaith, J., 1976. Load-displacement characteristics of articular cartilage. International Journal of Mechanical Sciences 18(9-10): 481-86.

Lanir, Y.,Fung, Y. C., 1974. Two-dimensional mechanical properties of rabbit skin. Ii. Experimental results. J Biomech 7(2): 171-82.

Little, J. P., Adam, C., Evans, J. H., Pettet, G. J.,Pearcy, M. J., 2007. Nonlinear finite element analysis of anular lesions in the l4/5 intervertebral disc. Journal of Biomechanics 40(12): 2744-2751.

Nusholtz, G. S., Bilkhu, S. M. F.,Uduma, K., 1996. Impact response of foam: The effect of the state of stress. Proceedings of the 40th Stapp Car Crash Conference, Albuquerque, New Mexico, Society of Automotive Engineers, Inc.

Sacks, M. S.,Sun, W., 2003. Multiaxial mechanical behavior of biological materials. Annu Rev Biomed Eng 5: 251-84.

Zeng, Y. J., Yager, D.,Fung, Y. C., 1987. Measurement of the mechanical properties of the human lung tissue. J Biomech Eng 109(2): 169-74.

TABLES

Table 1

Calibration results comparing the compressive force on the piston due to the fluid pressure and the force measured on the outer surface of the piston with a Hounsfield load cell

Compressive force on the piston due to fluid pressure (N)	Measured compressive force on the outer surface of the piston (N)	Calculated shear force (N)	Shear corrected measured force (N)	Absolute error between the force on the inner and outer piston surfaces (N)	Error relative to the fluid pressure force on the piston (%)
0	0.022	0	0.022	0.0217	
0.631	0.643	0.0074	0.636	0.0052	0.831
1.262	1.278	0.0147	1.264	0.0012	0.091
1.894	1.915	0.0221	1.893	0.0013	0.067
2.526	2.548	0.0295	2.519	0.0074	0.292
3.158	3.192	0.0368	3.155	0.0028	0.088
3.789	3.835	0.0442	3.791	0.0015	0.038
4.421	4.468	0.0516	4.417	0.0043	0.097
5.053	5.099	0.0589	5.040	0.0126	0.249
5.685	5.748	0.0663	5.682	0.0025	0.044
6.117	6.215	0.0713	6.144	0.0264	0.432
					Average = 0.223

FIGURES

Figure 1

CAD image of the biaxial compression rig. A. Assembled, B. With the lid and two sides removed, showing large discs representing the viewing windows in opposite walls, the attachment sights for the specimen on the intermediate walls and the specimen attached to nylon threads in the middle of the vessel, C. Specimen magnified

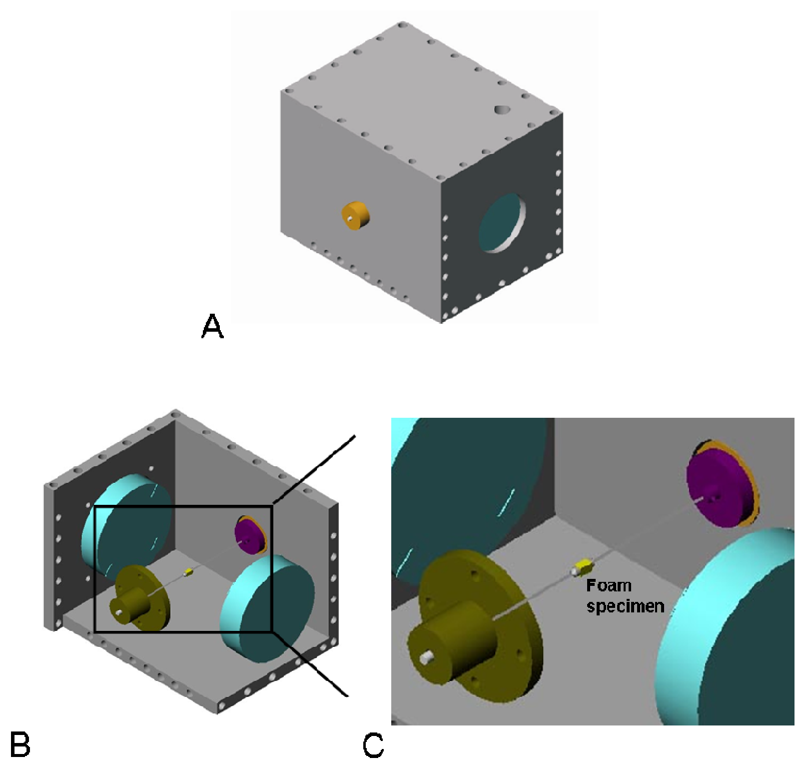


Figure 2

Principle of operation for the biaxial compression device. (Note: the specimen and piston size are exaggerated for illustrative purposes)

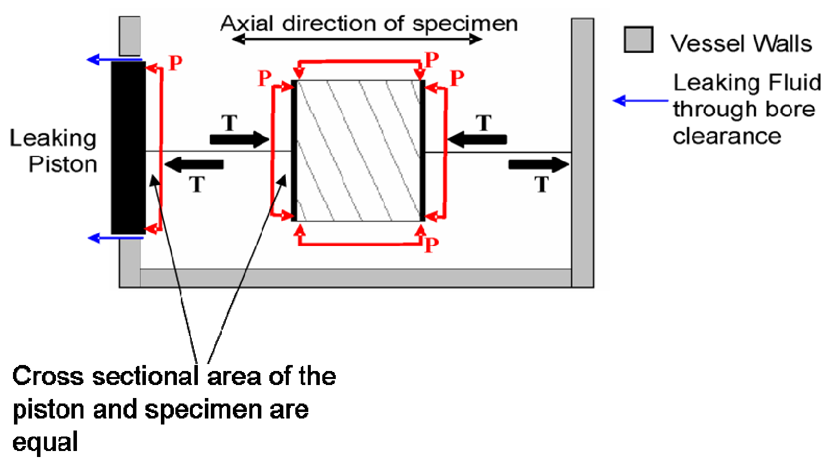


Figure 3

A. Ceramic piston (white) with titanium cap glued to the end. Using nylon thread, the titanium cap is attached to a dental cement plug, the end of which will be glued to the specimen; B. Schematic showing a cross section through the vessel wall (Hatching = wall, Dots = Bore insert with highly polished bore, Circles = ceramic piston); C. The ceramic piston located in the pressure vessel wall.

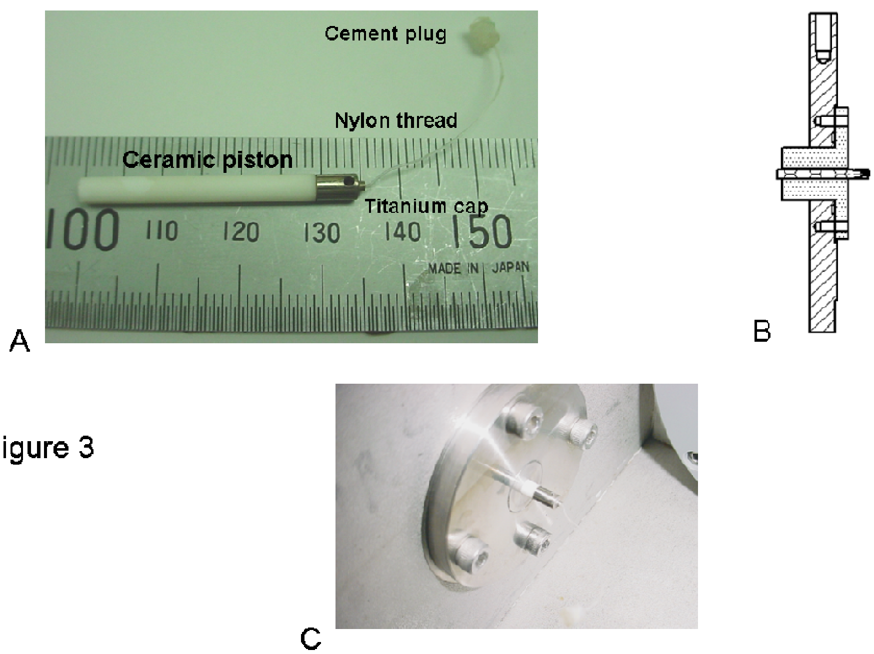
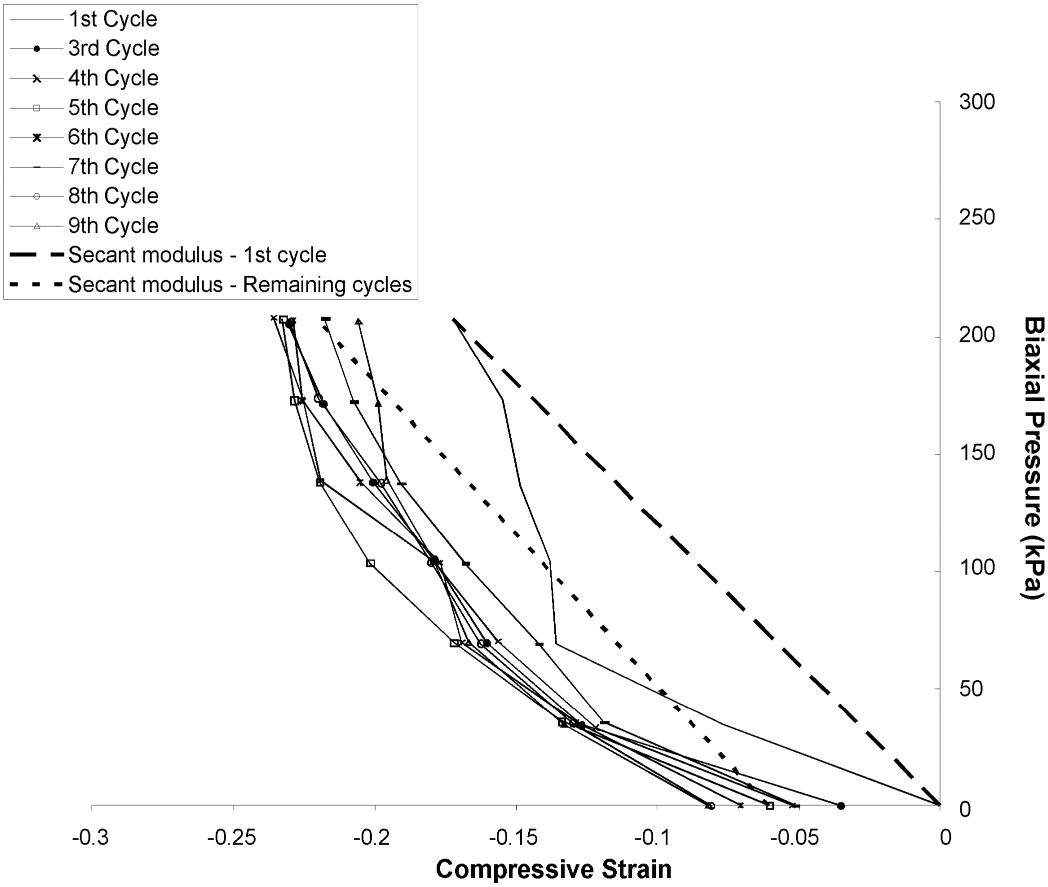


Figure 3

Figure 4

a. Biaxial pressure (kPa) vs. compressive strain and



b. Biaxial pressure (kPa) vs minimum measured deformed width (mm) of EVA foam during biaxial compression under repeated loading.